510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k041102

B. Purpose for Submission:
This is a new device.

C. Measurand:
Anti-nuclear antibodies.

D. Type of Test:
ELISA, semi-quantitative

E. Applicant:
IMMCO Diagnostics

F. Proprietary and Established Names:
ImmuLisa™ Anti-Nuclear Antibody (ANA) Screen ELISA

G. Regulatory Information:

1. Regulation section:
   21 CFR§866.5100, Antinuclear antibody immunological test system
2. Classification:
   Class II
3. Product Code:
   LJM, Antinuclear antibody (Enzyme-labeled) antigen, controls
4. Panel:
   82 Immunology

H. Intended Use:

1. Intended use(s):
   The ImmuLisa™ Anti-Nuclear Antibody (ANA) Screen ELISA is an enzyme linked immunosorbent assay for the detection of antinuclear and cytoplasmic antibodies in human serum to aid in the diagnosis of autoimmune diseases such as Systemic Lupus Erythematosus (SLE), Sjögren's Syndrome (SS), Mixed Connective Tissue Disease (MCTD) and Scleroderma.
2. Indication(s) for use:
   Same as intended use.
3. Special condition for use statement(s):
   The device is for prescription use only.
4. Special instrument Requirements:
   - Microplate reader capable of reading absorbance values at 450 nm. If dual wavelength is available, the reference filter should be set at 600-650 nm.
   - Automatic microplate washer capable of dispensing 200uL.

I. Device Description:
The IMMCO kit has a calibrator, a positive and a negative control, a microplate with individual breakaway microwells coated with antinuclear antigen, anti-human IgG conjugate, serum diluent, TMB enzyme substrate, stop solution and wash buffer. Except for the wash buffer, the reagents are ready to use.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   ImmuGlo™ Antinuclear Hep-2 cells IFA kit
2. Predicate K number(s):
   k883883
3. Comparison with predicate:

Similarities		
Item	**Device**	**Predicate**
	ImmuLisa ™ Antinuclear Antibody Screen	ImmuGlo™ Antinuclear Hep-2 IFA
Intended Use	ANA detection to aid in the diagnosis of autoimmune diseases	Same
Matrix	Serum	Same
Assay Type	Semi-quantitative	Same
Differences		
Item	**Device**	**Predicate**
Methodology	ELISA	Immunofluorescence
Screening dilution	1:101	1:40
Conjugate	Horseradish peroxidase	fluorescein
Antigen	Immobilized on the microwells	Immobilized on a glass slide

K. Standard/Guidance Document Referenced (if applicable):
None referenced.

L. Test Principle:
The ImmuLisa™ Anti-Nuclear Antibody (ANA) Screen is performed as solid phase immunoassay (ELISA). Microwells are coated with antigens from Hep-2 supplemented with other nuclear and cytoplasmic antigens, followed by blocking the unreacted sites to reduce nonspecific binding. Controls, calibrators and patient serum samples are incubated in the antigen coated wells which allows ANA present in the serum to bind. Unbound antibody and other serum proteins are removed by washing the microwells. Antibodies bound to the microwells are detected by adding enzyme labeled anti-human IgG conjugates to the wells. These enzyme conjugated antibodies bind specifically to the ANA attached to the antigen-coated wells. Unbound enzyme conjugate is removed by washing. Specific enzyme substrate (TMB) is then added to the wells and the presence of ANA is detected by a color change produced by the conversion of the substrate to a color product. The reaction is stopped and the intensity of the color change, which is proportional to the concentration of the antibody, is read by a spectrophotometer at 450 nm. Results are expressed in ELISA units per milliliter (EU/mL).

M. Performance Characteristics (if/when applicable):

1. Analytical performance:
   a. Precision/Reproducibility:

For the intra-assay and inter-assay precision studies, three samples with different concentrations of ANA were assayed in replicates of ten using two lots of plates. For the inter-assay studies, the assays were performed twice a day. All studies were performed by one operator.

The following results were obtained:

Sample (Mean EU/mL)	Inter-assay (Mean% CV)
19.5	5.2
51.4	6.2
107.7	6.4

Sample (Mean EU/mL)	Intra-assay (Mean %CV)
20.3	8.1
52.7	7.0
125.9	6.8

Reproducibility of the calibrator was determined by assaying the calibrator 10 times on one plate. The % CV of the calibrator in multiple assay-runs was 6.18%.

b. Linearity/assay reportable range:
Linearity was not determined as this assay does not use a standard curve.

Recovery study was performed by mixing three samples with known ANA levels with appropriate dilutions of another positive sample with known amounts of ANA. ANA levels of the three mixed samples were determined and the percent recoveries were calculated. Percent recoveries were 98.4, 100.1, and 111.4%.

c. Traceability (controls, calibrators, or method):
Since there is no reference material, the assay is calibrated in relative arbitrary units (EU/mL). Positive control and calibrator were derived from sera determined to be positive by immunofluorescence on Hep-2 obtained from various plasma centers. The sample was selected on the basis of the specific antibody reactivity and the concentration. For assignment of values, the samples were tested at various dilutions on two different lots of the ANA antigen coated plates.

d. Detection limit:
Not applicable.

e. Analytical specificity:
Interference Study:

Known positive samples were diluted in jaundiced, hemolytic, lipemic, and icteric sera and the levels of ANA were determined. The values obtained were compared with values of non-jaundiced, lipemic, hemolytic and icteric samples. The study demonstrates that lipemic, hemolytic, jaundice, and icteric sera have no significant effect on the assay.

Cross Reactivity Study:
Cross reactivity was studied in other autoimmune diseases. Samples from patients with Celiac Disease, Dermatitis Herpetiformis, and Pemphigus were obtained from clinical laboratories and tested on the IMMCO ANA screen ELISA. Results are summarized below:

Disease	# tested	#. Positive	% Positive
Celiac Disease	19	1	5%
Dermatitis herpetiformis	16	2	12%
Pemphigus	20	1	5%

The four samples found positive had low ANA concentrations of less than 40 EU/mL. ANA by indirect IFA has been reported to be positive in approximately 10% of the disease control and normal subjects (Kavanaugh, A. et al Arch: Pathol Lab Med 2000; 124: 71-81).

f. Assay cut-off:
The normal range was established by testing 67 serum samples from apparently healthy donors obtained from the local Red Cross. The cut-off value (mean plus 2 SD) was assigned an arbitrary unit value of 20 EU/mL. IMMCO determines the high point of the borderline zone to be 1.25 times greater or 5 EU/mL higher than the average plus two standard deviations, thus 20-25 EU/mL was set as borderline.

The following serves as a guide in interpretation of laboratory results. Each laboratory must determine its own normal values.

<20 EU/mL Negative
20-25 EU/mL Indeterminate (Borderline)
>25 EU/mL Positive

2. Comparison studies:
   a. Method comparison with predicate device:
   Two hundred ninety-two samples were tested with the IMMCO ANA Screen ELISA and the predicate device. Of the 292 samples, 107 were normal sera, 55 disease controls (19 celiac disease, 16 dermatitis herpetiformis, 20 pemphigus), 7 SLE patients, and 123 samples with different ANA specificities. The table below shows the comparison results.

	Hep-2 IFA		
IMMCO ANA Screen ELISA	Positive	Negative	Total
Positive	123	6	129
Negative	7	156	163
Total	130	162	292
Positive agreement	95%		
Negative agreement	96%		
Total Agreement	96%		

b. Matrix comparison:
Serum is the only recommended matrix

3. Clinical studies:
   a. Clinical sensitivity:
   Not provided.
   b. Clinical specificity:
   Not provided.
   c. Other clinical supportive data (when a and b are not applicable):
   Not applicable.
4. Clinical cut-off:
   See Assay cut-off
5. Expected values/Reference range:
   The expected values in a normal population are negative (<20 EU/mL) for adults and children. Some apparently healthy, asymptomatic individuals may test positive for ANA. The incidence of ANA in various disease groups can be referenced at Kavanaugh, A. et al (Arch Pathol Lab Med 2000; 124: 71-81).

N. Proposed Labeling:
The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:
The submitted material in this premarket notification is complete and supports a substantial equivalence decision.